



Setting up a Quantitative Analysis MS ChemStation

Getting Ready

1. Use the tutorial section "Quant Reports" on the MSD Reference Collection CD-ROM that came with your ChemStation software.
2. Know what compounds are to be quantitated.
3. Know what instrument conditions (heated zone temperatures, pressures, etc.) are to be used, i.e., set up an Acquisition method.
4. Know what target ion and qualifier ions (if any) are to be used for each compound (GC/MS only).

A target ion is an ion characteristic of the target compound, preferably one that distinguishes this compound from any others with similar retention times. The extracted ion chromatogram for this ion will be used for the quantitation.

Qualifier ions are selected from the mass spectrum of the target compound. The presence of these ions in the correct amounts relative to the target ion gives evidence of correct target compound identification. Depending on your choice of peak selection parameters, the qualifiers may influence how the software chooses between multiple "hits".

5. Prepare calibration sample(s).

A calibration sample is a sample containing a known concentration of the compound(s) to be quantitated (target compounds). It may also be referred to as a calibration standard or a standard mixture. If the method used an internal standard (ISTD), that compound must also be in the calibration sample.

6. Obtain a data file from the analysis of at least one of the above calibration samples in which the instrument conditions are those to be used in the quantitative analysis (e.g., the retention times are correct).

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A **quantitation database** is a collection of information and data needed to perform quantitative analysis. For each compound, the database includes the calibration table and the information required to extract and integrate the ion chromatograms. Calibration curves from the calibration tables are used to quantitate target compounds. The quantitation database is part of a method.

To Create a Quant Database

1. Load a calibration data file into Data Analysis (see Getting Ready). The TIC (total ion chromatogram) is automatically displayed.
2. Select **Calibrate / Set Up Quantitation** from the menu to define parameters that will apply to all compounds in the database. When you are finished, click **OK**.
3. When the Edit Compounds box appears, click on the position in the list of compounds where you want to insert a compound.

For example, to associate a compound with an internal standard already in the list, click on an entry below the internal standard. (If the list is empty i.e., you are just starting the database, click on **END OF COMPOUND LIST**.) Then click the **Insert Above** button.

4. The Quant Setup box appears beneath the display of the TIC or EIC. You will use this box to select target ions and qualifiers for each compound that you want to add to the database.

To Set Up a compound as a Quant Database Entry

The Quant Setup box lets you use the mouse to automatically enter a compound's retention time and target/qualifier ion masses and ratios into the database.

- A. Enter the name of the compound as you want it to appear in the database (and in reports).
- B. Note: Each compound in the database must have a unique name. Identical entries can cause compounds to be missed during quantitation. If you need to use the same name, because, for example, isomers of a compound elute at different times, use subtle variations such as Xylene and xylene (difference in capitalization).
- C. Check the **ISTD** box if the compound is an internal standard.
- D. Then select the **Tgt** button. The remaining fields in the box will be selected and filled in according to mouse actions on the display.
- E. Double-click the right mouse button on the scan of interest in the chromatogram. The selected spectrum will be displayed next to the Quant Setup box and the retention time will automatically be entered in the box.
- F. Note: In mixed mode quantitation, the spectrum may be selected from either the MS signal or the GC detector signal. If the selection is made from the MS signal, the quantitation signal will be an MS quantitation ion. If the selection is made from a GC signal, that signal will be used for quantitation.

- G. To select the target ion, move the mouse pointer to the target ion and click BOTH mouse buttons at the same time. The target ion mass will be entered in the box and Q1 qualifier ion button will be selected.
 - H. Note: The mouse pointer is shaped like a target sight when it is placed on the spectrum. The click selects the largest ion within an area that is about 1/30th of the width of the spectrum plot. Thus, you do not need to "aim" the mouse precisely. If, however, you want to select less abundant ion that is very close to a more abundant ion, you will need to zoom in on that part of the spectrum. Click and hold the left mouse button and drag the mouse to create a rectangle around the ions of interest. (This is especially important when choosing ions of halogenated compounds.)
 - I. To select a qualifier ion, click both mouse buttons on the ion. The mass will be entered in the box. Note that ratios to the target ion are also calculated and entered. (If you do not want to use qualifier ions, go to step J.)
 - J. Click the Save button to write the entry into the database.
 - K. To add another compound to the database, repeat steps A-J.
 - L. When you are finished adding compounds, click the Exit button.
5. When you are finished adding compounds, click the **Exit** button in the Quant Setup box. The Edit Compounds box is automatically displayed in case you want to view/edit the compounds in the database.
 6. Exit the Edit Compounds box. To generate response data for a calibration curve, select **Calibrate / Update/Update One Level**.
 7. Enter the concentration of the compound and the internal standard (if present). Then select **Add New Level** and enter a **Calib Level ID**. Click **Do Update**. The data file will be quantitated. (If you are asked whether to re-quant now, answer **yes**.)
 8. Again, the Edit Compounds box is automatically displayed in case you want to view/edit the concentration/response data on Page 3 of the compound entry.
 9. Add additional levels to the curve either by quantitating additional calibration files or by selecting **Calibrate / Update/ Quick Levels Update**, updating the information in the dialog box, and clicking **Do Update**.
 10. Save the database in a method. Be sure to do this before you leave Data Analysis. If you do not save, the information just entered into the database is lost.

Saving the Quant Database

Before leaving Data Analysis, save the quantitation database:

1. Select **Method / Save Method**.
2. The name of the current method is displayed in the Save Method dialog box. You may:
 - Keep the name of the current method.
 - Enter a different name.
3. Click **OK** to save the method, including the quantitation database, to the name specified.

When you leave Data Analysis, you will be reminded to save the changes:

- Click **Yes** if you want to save the changes and you will be prompted for the method name.
- Click **No** if you do not want to save the changes.

You are now ready to quantitate mass spectral data files containing unknown concentrations of one or more of the compounds in this database.

Reviewing the Quant Database

To review (or edit) the quantitation database, one compound at a time: Select **Calibrate / Edit Compounds**.

To look at the calibration curves, one compound at a time, click **Plot** when reviewing a compound entry. The display lists the concentration and response at all calibration levels and shows the correlation coefficient, type of fit, and the response for compounds not related to an internal standard. For compounds related to an internal standard, the concentration ratio and response ratio are plotted and listed instead of the concentration and response.

- Concentration ratio is Analyte Concentration / ISTD Concentration.
- Response ratio is Analyte Response / ISTD Response.

If any changes were made when reviewing the quant database, save the method again (see **Saving the Quant Database** above).

Quantitating Unknowns

After you have set up the quantitation database and calibration curves, you can analyze unknowns. This is done by acquiring and quantitating the unknowns using the Method you have calibrated in the steps above.

- Each compound to be quantitated must have a calibration curve with data from at least one calibration sample.
- The unknown sample must be analyzed under the same conditions used for the calibration samples.

When you are ready to prepare a report of the quantitative results, select **Quantitate / Generate Report**; this takes you to the Quant Report Options dialog box.

Generating a Quant Report

The Quant Report Options dialog box appears when you select **Quantitate / Generate Report** from the menu. Use this dialog box to specify the style and the destination for a quantitative report.

To generate a previously-calculated quant report interactively:

- Select **Quantitate / Generate Report** from the menu. The current data file is used.

To automatically generate a quant report as part of the current method (whenever the method is run, either using Run Method, or in a sequence):

- Select **Method / Edit Method**
- Select **Quant Report** in the Select Reports dialog box.
- Save the method or the change will not take effect and the report will not be generated automatically.

Most of the information in this document was taken from the on-line Help, which contains additional details on quantitation. To reach this part of the Help, select **Help ... Contents ... Analyzing data ... Using quantitation ... How to**. Then select the topic you want help on. Familiarity with the Help pages will also assist when troubleshooting quantitation problems.